0013-7227/02/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 87(2):650–654
Copyright © 2002 by The Endocrine Society

Role of Calpain-10 Gene Variants in Familial Type 2 Diabetes in Caucasians

STEVEN C. ELBEIN, WINSTON CHU, QIANFANG REN, CHRIS HEMPHILL, JOHN SCHAY, NANCY J. COX, CRAIG L. HANIS, AND SANDRA J. HASSTEDT

Division of Endocrinology (S.C.E., W.C., Q.R., C.H., J.S.), Department of Medicine, University of Arkansas for Medical Sciences and Central Arkansas Veterans Healthcare System, Little Rock, Arkansas 72205; Departments of Medicine and Human Genetics (N.J.C.), The University of Chicago, Chicago, Illinois 60637; Human Genetics Center (C.L.H.), The University of Texas Health Science Center at Houston, Houston, Texas 77225; and Department of Human Genetics (S.J.H.), University of Utah School of Medicine, Salt Lake City, Utah 84132

The calpain-10 gene (*CAPN10*) has been implicated in type 2 diabetes (T2DM) susceptibility by both linkage and association in a Hispanic population from Starr County Texas. Common intronic variants seem to alter CAPN10 mRNA levels and were associated with insulin resistance but not diabetes in Pima Indians. The role of these variants in Caucasian populations is less clear. We found some evidence for linkage of T2DM to chromosome 2q approximately 20 cM proximal to the *NIDDM1*/CAPN10 locus. To test the hypothesis that CAPN10 is a diabetes susceptibility locus in Caucasian families at high risk for T2DM, we examined the influence of the three previously implicated CAPN10 variants on both diabetes risk and measures of insulin sensitivity and glucose homeostasis. We genotyped approximately 700 members of 63 families for 3 variants (SNP-43, SNP-19, and SNP-63). We tested each variant separately and as haplotype combinations for altered transmission from parents to affected children (transmission disequilibrium test), and we tested for an effect of each variant individually on measures of glucose and insulin during a glucose tolerance test in nondiabetic family members. Finally, we looked for an effect of each variant on measures of insulin sensitivity (S_I) and insulin secretion estimated by frequently sampled iv glucose tolerance test and Minimal Model analysis. We could not confirm an increase in risk for T2DM susceptibility for any variant or for any haplotype combination, although we found marginal evidence for an increased risk of the 111/221 haplotype combination (*P* = 0.036) after ascertainment correction. However, both SNP-19 and SNP-63 increased fasting and/or postchallenge insulin levels, consistent with reduced insulin sensitivity. Furthermore, SNP-19 had modest effects on insulin sensitivity measured by homeostatic model, and on postchallenge glucose. The reduction in insulin sensitivity was confirmed by analysis of the subset of individuals who underwent iv glucose tolerance tests, where SNP-19 significantly altered the insulin sensitivity index. CAPN10 cannot be considered a major diabetes susceptibility gene in our population and seems unlikely to explain the observed linkage findings. However, CAPN10 influences insulin sensitivity and glucose homeostasis in nondiabetic members of kindreds at high risk for T2DM. (*J Clin Endocrinol Metab* 87: 650–654, 2002)

TWIN AND FAMILY studies provide convincing evidence that type 2 diabetes (T2DM) is strongly inherited, with an increased risk to the sibling of a diabetic individual that is at least 3-fold higher than the population at large among individuals of European ancestry (1). Nonetheless, genes for common T2DM have been elusive, probably because the disease is heterogeneous and multiple loci contribute to diabetes susceptibility in concert with environmental factors such as diet and activity. Recent studies have identified likely susceptibility loci on chromosomes 2q (*NIDDM1*) (2), 1q (3–5), and 20 (6–8), among others. Of these loci, only *NIDDM1* has been mapped to a single gene, calpain-10 (*CAPN10*). *CAPN10* is a ubiquitously expressed member of the calpain-like cysteine protease family (9). The risk of T2DM in Hispanic individuals at this locus can be accounted for by three variants, SNP-19, SNP-43, and SNP-63, which reside in introns of the *CAPN10* gene. Heterozygosity for two haplotypes was associated with the highest risk for T2DM in Hispanic, German, and Finnish populations in the original observation, but this combination is unusual in Caucasian populations, and significance is thus uncertain. *In vitro* data suggested that SNP-43 altered *CAPN10* expression, and the G/G genotype for SNP-43 also reduced *CAPN10* messenger RNA in muscle biopsy specimens in Pima Indians (10).

We have extensively characterized a cohort of multigenerational families of Northern European ancestry ascertained in Utah for at least two diabetic siblings. The most significant locus identified in these families maps to the ApoA2 region of chromosome 1q21-q23 (4). Although we found no evidence for linkage of T2DM to the *NIDDM1* region of chromosome 2q, we did find suggestive linkage to a region approximately 15–20 cM proximal to *CAPN10* (logarithm of odds = 2.180 on affected sib pair analysis). Because replication of linkage even in the same population may occur this far from the initial observation (11), this observation might also support a role for *CAPN10* in susceptibility to T2DM in Caucasians.

To examine the role of *CAPN10* in Utah families, we typed

Abbreviations: AIR_g, Acute insulin response to glucose; BMI, body mass index; *CAPN10*, calpain-10 gene; CI, confidence interval; DI, disposition index; FSIGT, frequently sampled iv glucose tolerance test; HOMA, homeostasis model assessment; OGTT, oral glucose tolerance test; OR, odds ratio; S_I, insulin sensitivity index; τ, proportion of transmitted alleles; TDT, transmission disequilibrium test; T2DM, type 2 diabetes.

approximately 700 members of 63 families ascertained for Northern European ancestry and at least 2 siblings with T2DM. We have examined the association of each variant with T2DM, both individually and after assigning haplotypes, using the transmission disequilibrium test (TDT) (12). We have also tested for an effect of each trait on glucose and insulin measures available from most nondiabetic family members, and on insulin sensitivity (S_I) measures available in a subset of family members.

Subjects and Methods

Subjects

Families were ascertained for at least 2 siblings who were diagnosed with T2DM before age 65 yr. At most, one parent was known to have T2DM. All subjects were of Northern European ancestry. All available parents and siblings of the index sib pair were studied, as were any available offspring of diabetic siblings. Details of the study population have been described previously (4, 13). The total study population comprised 854 available individuals from 63 kindreds. Genotypic data were available for 707 people for SNP-43, 700 individuals for UCSNP19, and 669 people for SNP-63. Each participant in the study gave informed consent under a protocol approved by the Institutional Review Board of the University of Utah Health Sciences Center, Salt Lake City. All clinical studies were performed at the University of Utah General Clinical Research Center.

Genotypic analysis

Initial typing of SNP43 was done by PCR-restriction fragment length polymorphism with enzyme Nsi I (9, 14). After typing the entire pedigree membership twice and identifying obligate recombination events between closely spaced polymorphisms, we determined that our initial SNP typing was not accurate, and we retyped the full pedigree set using an oligonucleotide ligation assay. SNP-19 was typed with upstream primer GTTTGGTTCTCTTCAGCGTGGAG and downstream primer CATGAACCCTGGCAGGGTCTAAG. Products of 187 bp and 155 bp were separated on 2% agarose gels, visualized with ethidium bromide, and scored either by hand or semiautomatically using GeneProfiler software (Scanalytics, Inc.; Fairfax, VA) after capturing the gels as digital images using a UV transilluminator and Eagle Eye II (Stratagene, La Jolla, CA). Blinded duplicates were included in all assays.

Final typing for SNP-43 and SNP-63 was performed using an oligonucleotide ligation assay. All primers were designed from published *CAPN10* sequence (9). Amplification primers were CTCTGATTCCCATGGTCTGTAG and CACGCTTGCTGTGAAGTAATGC (SNP-43), and CTCAGCACCCAGTCCTACCA and GAGCAAATAAGGCGCAGGT′ (SNP63). For SNP-43, allele specific primers were TCACGCTTGCTGCGAAGTAAGGC*G* and TTTTTCACGCTTGCTGCGAAGTAAGGC*A*. The common SNP-43 oligonucleotide was TTTGAAGGTGAGGCTAAGCCTTGACTTGGTGAGGATGGTCGTTTTACAACGTCGTG. For SNP-63, the allele specific oligonucleotides were CAGGGCCTGACGGGGGTGGAGC and TTTTCAGGGCCTGACGGGGGTGGAG*T*, and the common oligonucleotide was GAGGGGGTGGGCCGCGTCTGTGCAGGC. Allele-specific primers were labeled with γ ^{32}P-deoxy-ATP (6000 Ci/mmol; NEN Life Science Products, Boston, MA) using T_4 polynucleotide kinase (New England Biolabs, Inc., Beverly, MA). Samples were heated to 99 C for 30 min to inactivate *Taq* polymerase before ligation. Oligonucleotide ligations were carried out for 20 cycles of 94 C denaturation (20 sec) and 45 C ligation (1 min) using *Taq* DNA ligase (New England Biolabs, Inc.). Samples were separated on 8% polyacrylamide gels containing 8 M urea and imaged on a Storm 860 Phosphoimager (Molecular Dynamics, Inc., Sunnyvale, CA). Semiautomated scoring was performed using GeneProfiler on digitized images.

Statistical analysis

Likelihoods of the genetic models (15) were computed using the Pedigree Analysis Package (16) and the maxima were obtained using GEMINI (17). Hypothesis testing was performed by comparing the likelihood of a submodel to the likelihood of a more general model. Twice the natural logarithm of the ratio of the likelihoods has an asymptotic χ^2 distribution. The allele frequency of each SNP was estimated from unrelated pedigree members, assuming Hardy-Weinberg equilibrium but using genotypes on all pedigree members to provide information when individuals had not been genotyped. Haplotype frequencies were similarly estimated assuming tight linkage between the SNPs. We computed the probability of each genotype for an individual as the relative likelihood conditional on assignment of that genotype to the individual. The TDT (12) estimated the probability that a heterozygous parent transmitted each allele or haplotype to an affected offspring more often than expected by chance. Significance was tested through comparison of the likelihood with the estimated transmission probability to the likelihood with the transmission probability fixed at 50%. The odds ratio (OR) for each genotype was estimated relative to all other genotypes, similar to penetrance estimation within pedigrees. To correct the ORs for family membership, we estimated ORs of each genotype or haplotype combination against all other combinations after correcting for familial correlations and ascertainment.

For quantitative trait analyses, we tested only nondiabetic individuals. Insulin and glucose measures were log-transformed to normality and adjusted for age and gender. Mean trait values for heterozygous individuals were constrained to fall between mean values for homozygous individuals. For analysis among obese individuals, we defined obesity as body mass index (BMI) greater than 27.2 kg/m^2 for men and BMI greater than 26.9 kg/m^2 for women (18). Analysis of individuals who had undergone frequently sampled iv glucose tolerance tests (FSIGT) was done using mixed-effects linear regression analysis in SPSS, Inc. v 10.1 (SPSS, Inc., Chicago, IL). Family membership was a random factor, genotype and diagnosis (normal and impaired glucose tolerance) were fixed factors, and age and ln-transformed BMI were covariates, as we have described previously (19, 20). BMI, S_I, acute insulin response to glucose (AIR_g), and disposition index (DI) were all ln-transformed before analysis. This analysis provides results comparable with that conducted in Pedigree Analysis Package to account for exact pedigree relationships (19). All results are presented without correction for multiple testing, because we were testing prior hypotheses.

Results

The frequency of allele 1 for each SNP in our population was 0.74 ± 0.02 for SNP-43, 0.43 ± 0.02 for SNP-19, and 0.92 ± 0.01 for SNP-63. These results were similar to those observed by Horikawa *et al.* (9) in European populations. The frequency of the predicted haplotypes is shown in Table 1. Of the 804 predicted haplotypes, 89.4% were predicted with 90% or greater certainty, and 82.6% could be predicted with absolute certainty. We tested for a deviation from equal transmission of each allele from parent to affected offspring for each SNP. In contrast to our preliminary report based on restriction typing of SNP-43, we found no significant deviation from equal transmission of alleles to affected offspring for any SNP [proportion of alleles transmitted (τ) = 0.45, τ = 0.47, and τ = 0.53 for allele 1 for SNP-43, SNP-19, and SNP-63, respectively; $P > 0.20$ for all]. Transmission from males to affected offspring or females to affected offspring likewise

TABLE 1. Estimated frequency of each *CAPN10* haplotype in Utah family members

SNP 43 allele	SNP 19 allele	SNP 63 allele	Frequency	Transmission
1	2	1	0.31	0.50
1	2	2	0.01	0.46
1	2	2	0.35	0.46
1	1	2	0.07	0.36
2	2	1	0.25	0.18
2	2	2	0.00	
2	1	1	0.01	0.57
2	1	2	0.00	

did not differ significantly from τ = 0.5 for any individual SNP, nor did transmission of haplotypes differ significantly from 0.5 (χ^2 = 5.84, P = 0.21). The effective number of transmissions from heterozygous parents to affected offspring tested was 180 for SNP-43, 190 for SNP-19, and 50 for SNP-63. Based on the number of transmissions, the deviation from 50% transmission (τ = 0.5) to detect significant transmission disequilibrium at $P < 0.05$ would be 0.57 for SNP-43 and SNP-19, and 0.64 for SNP-63.

Specific haplotype combinations have been suggested to account for the increased risk of CAPN10 (9); to test this hypothesis, we examined the risk of T2DM by haplotype combination (genotype), as shown in Table 2. After correcting ORs for ascertainment as well as familial correlations, only the 111/221 haplotype combination significantly increased risk (OR,1.48; χ^2 = 4.38, P = 0.036), with a confidence interval (CI) of 1.06–1.91. Whereas the 111/111 genotype was marginally protective before ascertainment correction, it failed to approach significance when the ascertainment correction was included (risk, 0.69, χ^2 = 1.95, P = 0.162). The risk of T2DM with the 112/121 haplotype combination, which contributed the highest risk in Hispanic subjects (9), was not significantly different from 1.00 in our population and, if anything, tended to be protective (OR, 0.67). However, this haplotype combination is only predicted to represent 2.1% of Utah individuals; and consequently, our power to detect an effect of the magnitude previously described in Hispanic subjects was limited. Although the 111/221 haplotype seemed to significantly increase the risk of T2DM, this finding was true only after correcting for ascertainment and without correction for multiple analyses.

We next tested the effects of each *CAPN10* SNP on quantitative traits measured during the oral glucose tolerance test (OGTT) and related to glucose homeostasis, including glucose and insulin. The significant results are shown in Table 3. SNP-43 approached significance for fasting glucose (χ^2 = 5.27, P = 0.07) when no specific genotype was tested. No other effect on glucose or insulin levels during the OGTT was found. When we tested the specific recessive hypothesis (SNP-43 1 1 or GG genotype against 1 2 or GA and 2 2 or AA), SNP-43 significantly, but marginally, increased the mean glucose from 4.7 mM to 4.9 mM (P = 0.035). SNP-19 showed significant effects on fasting insulin (χ^2 = 6.62, P = 0.04), 30-min glucose (χ^2 = 6.16, P = 0.045), 60-min glucose (χ^2 = 8.74, P = 0.013), and insulin resistance by homeostasis model assessment (HOMA) (χ^2 = 6.70, P = 0.035) (Table 3). The effect on fasting insulin was most marked among obese individuals (χ^2 = 10.20, P = 0.0061; data not shown). Similarly, SNP-63 showed significant effects on total insulin area under the curve (χ^2 = 3.83, P = 0.05), 90-min insulin (χ^2 = 5.41, P = 0.020), and 2-h insulin (χ^2 = 5.37, P = 0.021). Estimated genotypic means for significant associations with quantitative traits are shown in Table 3.

Finally, we examined the effects of *CAPN10* variants on S_I determined by minimal model analysis of FSIGT data (21), insulin secretion determined as the AIR_g, and the index of β-cell compensation (DI). Genotypic data were available for 117/128 possible individuals. SNP-19 was a significant determinant of S_I (P = 0.017), with most significance noted between individuals homozygous for the deletion allele, who had a higher S_I, and all others (P = 0.016). Marginal means are shown in Table 4. In contrast, neither SNP-43 nor SNP-63 genotype significantly influenced S_I, even when SNP-43 was considered to be recessive (P = 0.4). None of the *CAPN10* variants had a significant effect on insulin secretion (AIR_g or DI).

TABLE 2. Risk of T2DM by haplotype combination

Haplotype combination	Risk (OR)	95% CI
111/111	0.69[a]	0.36–1.02
111/121	1.12	0.81–1.43
111/112	1.39	0.61–2.17
111/221	1.48[b]	1.06–1.91
112/112		
112/121	0.67	0.18–1.15
112/221	1.52	0.60–2.43
121/121	0.65[c]	0.30–1.00
121/221	0.80	0.48–1.11
221/221	0.96	0.46–1.45

Risk of T2DM (OR) and 95% CI are shown for each haplotype combination of alleles from SNP-43, SNP-19, and SNP-63, after Ref. 9. ORs are corrected for ascertainment and familial correlations.

[a] χ^2 = 1.95, P = 1.62.

[b] χ^2 = 4.38, P = 0.036.

[c] χ^2 = 2.09, P = 0.148.

Discussion

CAPN10 was first implicated in T2DM susceptibility by linkage in Hispanics from Starr County, Texas (2), and subsequently identified through linkage disequilibrium studies (9). In Hispanics, Horikawa *et al.* (9) estimated the population-attributable risk at 14%, whereas the risk in Caucasians was estimated at only 4%. Although Horikawa *et al.* found the same risk haplotypes among Caucasians from Botnia; Finland; and Dresden, Germany, as in Hispanics (112/121), no haplotype combination was significant in the Caucasian populations individually. Likewise, SNP-43 was not significantly associated with T2DM in Pima Indians (10). Similarly, none of the three SNPs tested here were associated with T2DM in a recent study of 743 sib pairs from the United Kingdom (22). In contrast, a combined analysis of both Botnia and Dresden Caucasian populations suggested an OR of 3.16, with a 95% CI from 1.19–8.40. Additional data supporting a role for *CAPN10* variants includes evidence for *in vitro* effects of SNP-43 on gene expression (transcription) and on binding of nuclear extracts (9). Among euglycemic Pima Indians, homozygosity for the G allele of SNP-43 resulted in higher fasting glucose, higher 2-h insulin, decreased glucose oxidation, and decreased glucose disposal (10). Individuals with the SNP-43 G/G (1 1) genotype also showed 53% lower muscle *CAPN10* mRNA levels.

In the present study, we successfully genotyped at least 669 individuals from 63 multigenerational families of Northern European descent, and we used a family-based approach to test the role of *CAPN10* using the 3 variants to which most of the risk has been attributed (9). We did not find evidence for increased transmission of any allele for these 3 variants, and we found only marginal evidence for an increased risk of the 111/221 haplotype combination. Because we tested multiple haplotype combinations, and this was not the combination previ-

TABLE 3. Genotypic means for quantitative traits associated with CAPN10 variants

Trait	SNP	11	12	22	*P* value
Fasting insulin (pmol/liter)	SNP-19	74	80	98	0.04
Insulin AUC (pmol-min/liter)	SNP-63	39,006	50,616	50,616	0.05
90-min insulin (pmol/liter)	SNP-63	365	524	524	0.02
120-min insulin (pmol/liter)	SNP-63	374	529	529	0.02
Fasting glucose (mmol/liter)	SNP-43	4.9	4.7	4.7	0.035
30-min glucose (mmol/liter)	SNP-19	8.5	7.9	7.9	0.045
60-min glucose (mmol/liter)	SNP-19	7.9	6.9	6.9	0.013
HOMA-IR	SNP-19	1,150	1,216	1,204	0.035

Values are estimated from maximum likelihood analysis of full family data after log-transformation to normality and adjustment for age and gender (see *Subjects and Methods*).

IR, Insulin resistance.

TABLE 4. Effect of SNP-19 on S_I

Genotype	Number	Mean	95% CI
11	29	7.05	5.04, 9.86
12	70	6.32	5.07, 7.89
22	15	10.70	6.86, 16.18

Values of S_I are given for each SNP-19 genotype in 114 individuals who underwent frequently sampled iv glucose tolerance testing. Estimated marginal means from in-transformed values and the 95% CI values on the logarithmic scale were converted back to standard units. S_I is given as 10^{-5} min^{-1}/[pmol/liter]. As stated in the text, the differences are significant at $P = 0.017$.

ously shown to cause the highest risk, this finding must be interpreted with caution. Our results are consistent with the observations in the individual Botnia, Dresden, and United Kingdom populations and the results for SNP-43 in Pima Indians. Three possible explanations reconcile our findings with those of Horikawa *et al.* (9). First, the high-risk haplotype combination, and particularly the 112 haplotype, is relatively rare among Caucasian populations; and thus, both the population attributable risk and our power to detect a significant effect are lower than in Hispanic individuals. Second, we have used TDT as applied to families with multiple affected individuals. Though the specific power of our method, compared with other TDT methods (20, 23–26), is unknown, in the absence of population stratification, TDT is probably less powerful than the case control design (27, 28). We would be unable to detect excess transmission less than 57% for SNP-19 or SNP-43, and less than 64% for SNP-63. Thus, our failure to detect transmission disequilibrium for *CAPN10* variants in the Utah population is consistent with a small effect of these *CAPN10* variants on diabetes risk in this population and the low frequency of the highest risk haplotypes in Caucasians. Finally, the highest risk among Hispanic individuals was for the combination of haplotypes 112/121, rather than for individual haplotypes. TDT, which tests preferential transmission of a single haplotype, is not well suited to test models in which a specific haplotype combination confers the increased risk.

In contrast to our failure to confirm an association of *CAPN10* variants with T2DM, we do confirm the findings of Baier *et al.* (10) and extend their results to an effect of the SNP-19 variant and to a Caucasian population. Although our data confirm that the SNP-43 GG (allele 1 in Tables 1–3) genotype very slightly raises fasting glucose in nondiabetic Caucasian individuals from families with a strong predisposition to T2DM, this finding is marginal and of uncertain significance. However, we find significant effects of SNP-19 on fasting insulin and HOMA-derived insulin sensitivity (29), effects of SNP-19 on postchallenge glucose levels, and significant effects of SNP-19 on S_I from the FSIGT in a subset of individuals. Thus, like Baier *et al.* (10), we show significant effects of *CAPN10* on two measures of insulin sensitivity, albeit with SNP-19 rather than SNP-43. The effects of SNP-63, which is relatively rare in our population, on postchallenge insulin and insulin area under the curve, further support a CAPN10 role in insulin sensitivity. Finally, we suggest an interaction of *CAPN10* variants and obesity in determining insulin sensitivity. That interaction might explain our failure to find an effect of SNP-43 on insulin sensitivity because BMI was a covariate in those analyses. Nonetheless, all of these effects are modest. Furthermore, because we tested a prior hypothesis, we did not correct for multiple testing. One might argue that correction for multiple testing would negate the modest *P* values found for our associations ($P > 0.01$ for most analyses). Additional studies in multiple populations are required, to address this concern.

A recent study suggested that calpains may regulate insulin secretion (30). We found effects on neither $AIR_{glucose}$ nor DI in individuals who had undergone iv glucose tolerance testing, and no effect on the 30-min insulin during the OGTT. Thus, we were unable to demonstrate a role of these CAPN10 SNPs in insulin secretion. Recently, Stumvoll *et al.* (31) showed increased insulin secretion in German subjects who carried the SNP-43 GG genotype, using a hyperglycemic clamp study. That increase would seem to be the opposite of the expected effects to increase diabetes risk.

We have previously demonstrated suggestive linkage to chromosome 2q somewhat proximal to *CAPN10* (4). Although *CAPN10* variants clearly influence fasting glucose and insulin sensitivity among nondiabetic family members, our inability to demonstrate a convincing association of specific *CAPN10* alleles with T2DM suggests that this finding of linkage probably cannot be attributed to *CAPN10*. No analysis in this region proximal to *CAPN10* has reached genome-wide significance; and thus, evidence for linkage may be spurious. However, our results correspond to a similar peak in other studies (2) and may suggest a second susceptibility locus on chromosome 2. We are currently pursuing this possibility.

In conclusion, our results support *CAPN10* as an influence on insulin sensitivity and fasting glucose as one of several loci influencing the risk of T2DM. However, this increased risk in our families, which were ascertained for a strong history of T2DM, seems to be modest. At least one other major susceptibility locus seems to segregate (4) in these families, and this locus (along with power considerations discussed above) likely

obscures the influence of *CAPN10* on T2DM. Other very common variants also seem to modestly alter diabetes risk (32–34), thus contributing the picture of a disease influenced by multiple common variants that differ in the degree of increased risk in different populations. The presence of multiple susceptibility and protective variants for complex diseases is perhaps not surprising, and this picture promises to become more complicated as susceptibility genes implicated in other genome scans are identified. Interestingly, we now report on a second population in which a biological effect of the *CAPN10* variants can be demonstrated despite finding evidence for neither linkage nor association.

Acknowledgments

Pedigree sampling, oral glucose tolerance testing, and iv glucose tolerance testing were performed at the General Clinical Research Center of the University of Utah. We thank Kim Wegner and Cindy Miles for assistance in subject recruitment, and Teresa Maxwell for DNA preparation.

Received July 9, 2001. Accepted October 22, 2001.

Address all correspondence and requests for reprints to: Steven C. Elbein, M.D., Endocrinology 111J/LR, John L. McClellan Memorial Veterans Hospital, 4700 West 7th Street, Little Rock, Arkansas 72205. E-mail: elbeinstevenc@uams.edu.

This work was supported by NIH Grant DK-39311 (to S.C.E.) and Grants DK-55889, DK-20595, and DK-47486 (to N.J.C.); by the Research Service of the Department of Veterans Affairs; by Public Health Service Grant M01-RR-00064 from the National Center for Research Resources; and by a Genetics of NIDDM Family Acquisition Grant from the American Diabetes Association.

References

1. **Rich SS** 1990 Mapping genes in diabetes: genetic epidemiological perspective. Diabetes 39:1315–1319
2. **Hanis CL, Boerwinkle E, Chakraborty R, Ellsworth DL, Concannon P, Stirling B, Morrison VA, Wapelhorst B, Spielman RS, Gogolin-Ewens KJ, Shepard JM, Williams SR, Risch N, Hinds D, Iwasaki N, Ogata M, Omori Y, Petzold C, Rietzch H, Schroder HE, Schulze J, Cox NJ, Menzel S, Boriraj VV, Chen X, Lim LR, Linder T, Mereu LE, Wang Y-Q, Kiang K, Yanagata K, Yang Y, Bell GD** 1996 A genome-wide search for human non-insulin-dependent (type 2) diabetes genes reveals a major susceptibility locus on chromosome 2. Nat Genet 13:161–166
3. **Hanson RL, Ehm MG, Pettitt DJ, Prochazka M, Thompson DB, Timberlake D, Foroud T, Kobes S, Baier L, Burns DK, Almasy L, Blangero J, Garvey WT, Bennett PH, Knowler WC** 1998 An autosomal genomic scan for loci linked to type II diabetes mellitus and body-mass index in Pima Indians. Am J Hum Genet 63:1130–1138
4. **Elbein SC, Hoffman MD, Teng K, Leppert MF, Hasstedt SJ** 1999 A genome-wide search for type 2 diabetes susceptibility genes in Utah Caucasians. Diabetes 48:1175–1182
5. **Vionnet N, Hani E-H, Dupont S, Gallina S, Francke S, Dotte S, De Matos F, Durand E, Lepretre F, Lecoeur C, Gallina P, Zekiri L, Dina C, Froguel P** 2000 Genome-wide search for type 2 diabetes-susceptibility genes in French Whites: evidence for a novel susceptibility locus for early-onset diabetes on chromosome 3q27-qter and independent replication of a type 2-diabetes locus on chromosome 1q21–q24. Am J Hum Genet 67:1470–1480
6. **Bowden DW, Sale M, Howard TD, Qadri A, Spray BJ, Rothschild CB, Akots G, Rich SS, Freedman BI** 1997 Linkage of genetic markers on human chromosomes 20 and 12 to NIDDM in Caucasian sib pairs with a history of diabetic nephropathy. Diabetes 46:882–886
7. **Ghosh S, Watanabe RM, Valle TT, Hauser ER, Magnuson VL, Langefeld CD, Ally DS, Mohlke KL, Silander K, Kohtamaki K, Chines P, Balow Jr J, Birznieks G, Chang J, Eldridge W, Erdos MR, Karanjawala ZE, Knapp JI, Kudelko K, Martin C, Morales-Mena A, Musick A, Musick T, Pfahl C, Porter R, Rayman JB, et al.** 2000 The Finland-United States Investigation of Non-Insulin-Dependent Diabetes Mellitus Genetics (FUSION) study. I. An autosomal genome scan for genes that predispose to type 2 diabetes. Am J Hum Genet 67:1174–1185
8. **Ji L, Malecki M, Warram JH, Yang Y, Rich SS, Krolewski AS** 1997 New susceptibility locus for NIDDM is localized to human chromosome 20q. Diabetes 46:876–881
9. **Horikawa Y, Oda N, Cox NJ, Li X, Orho-Melander M, Hara M, Hinokio Y, Lindner TH, Mashima H, Schwarz PE, del Bosque-Plata L, Horikawa Y, Oda Y, Yoshiuchi I, Colilla S, Polonsky KS, Wei S, Concannon P, Iwasaki N, Schulze J, Baier LJ, Bogardus C, Groop L, Boerwinkle E, Hanis CL, Bell GI** 2000 Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. Nat Genet 26:163–175
10. **Baier LJ, Permana PA, Yang X, Pratley RE, Hanson RL, Shen GQ, Mott D, Knowler WC, Cox NJ, Horikawa Y, Oda N, Bell GI, Bogardus C** 2000 A calpain-10 gene polymorphism is associated with reduced muscle mRNA levels and insulin resistance. J Clin Invest 106:R69–R73
11. **Roberts SB, MacLean CJ, Neale MC, Eaves LJ, Kendler KS** 1999 Replication of linkage studies of complex traits: an examination of variation in location estimates. Am J Hum Genet 65:876–884
12. **Ewens WJ, Spielman RS** 1995 The transmission/disequilibrium test: history, subdivision, and admixture. Am J Hum Genet 57:455–464
13. **Elbein SC, Hasstedt SJ, Wegner K, Kahn SE** 1999 Heritability of pancreatic beta-cell function among nondiabetic members of Caucasian familial type 2 diabetic kindreds. J Clin Endocrinol Metab 84:1398–1403
14. **Ren Q-F, Hasstedt S, Hanis C, Elbein SC** 2000 Increased transmission of NIDDM1 variant in Caucasian familial type 2 diabetes. Diabetes 49(Suppl 1):A200
15. **Elston RC, Stewart J** 1971 A general model for the genetic analysis of pedigree data. Hum Hered 21:523–542
16. **Hasstedt SJ** 2001 PAP: Pedigree Analysis Package, V5. Salt Lake City: University of Utah, Department of Human Genetics
17. **Lalouel JM** 1979 GEMINI-a computer program for optimization of general nonlinear functions. 14. Salt Lake City: University of Utah, Department of Medical Biophysics and Computing. Technical Report
18. **Burton BT, Foster WR, Hirsch J, Van Itallie TB** 1985 Health implications of obesity: an NIH Consensus Development Conference. Int J Obes 9:155–170
19. **Elbein SC, Sun J, Scroggin E, Teng K, Hasstedt SJ** 2001 Role of common sequence variants in insulin secretion in familial type 2 diabetic kindreds: the sulfonylurea receptor, glucokinase, and hepatocyte nuclear factor 1alpha genes. Diabetes Care 24:472–478
20. **Allison DB, Heo M, Kaplan N, Martin ER** 1999 Sibling-based tests of linkage and association for quantitative traits. Am J Hum Genet 64:1754–1763
21. **Pacini G, Bergman RN** 1986 MINMOD: a computer program to calculate insulin sensitivity and pancreatic responsivity from the frequently sampled intravenous glucose tolerance test. Comput Methods Programs Biomed 23:113–122
22. **Evans JC, Frayling TM, Cassell PG, Saker PJ, Hitman GA, Walker M, Levy JC, O'Rahilly S, Rao PV, Bennett AJ, Jones EC, Menzel S, Prestwich P, Simecek N, Wishart M, Dhillon R, Fletcher C, Millward A, Demaine A, Wilkin T, Horikawa Y, Cox NJ, Bell GI, Ellard S, McCarthy MI, Hattersley AT** 2001 Studies of association between the gene for calpain-10 and type 2 diabetes mellitus in the United Kingdom. Am J Hum Genet 69:544–552
23. **Boehnke M, Langefeld CD** 1997 A transmission/disequilibrium test that uses both affected and unaffected offspring. Am J Hum Genet 61(Suppl):A269
24. **Clayton D** 1999 A generalization of the transmission/disequilibrium test for uncertain-haplotype transmission. Am J Hum Genet 65:1170–1177
25. **Spielman RS, Ewens WJ** 1998 A sibship test for linkage in the presence of association: the sib transmission/disequilibrium test. Am J Hum Genet 62: 450–458
26. **Horvath S, Laird NM** 1998 A discordant-sibship test for disequilibrium and linkage: no need for parental data. Am J Hum Genet 63:1886–1897
27. **Risch N, Teng J** 1998 The relative power of family-based and case-control designs for linkage disequilibrium studies of complex human diseases I. DNA pooling. Genome Res 8:1273–1288
28. **Risch NJ** 2000 Searching for genetic determinants in the new millennium. Nature 405:847–856
29. **Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC** 1985 Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412–419
30. **Sreenan SK, Zhou YP, Otani K, Hansen PA, Currie KP, Pan CY, Lee JP, Ostrega DM, Pugh W, Horikawa Y, Cox NJ, Hanis CL, Burant CF, Fox AP, Bell GI, Polonsky KS** 2001 Calpains play a role in insulin secretion and action. Diabetes 50:2013–2020
31. **Stumvoll M, Fritsche A, Madaus A, Stefan N, Weisser M, Machicao F, Haring H** 2001 Functional significance of the UCSNP-43 polymorphism in the CAPN10 gene for proinsulin processing and insulin secretion in nondiabetic Germans. Diabetes 50:2161–2163
32. **Altshuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl MC, Nemesh J, Lane CR, Schaffner SF, Bolk S, Brewer C, Tuomi T, Gaudet D, Hudson TJ, Daly M, Groop L, Lander ES** 2000 The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. Nat Genet 26:76–80
33. **Hegele RA, Harris SB, Zinman B, Wang J, Cao H, Hanley AJ, Tsui LC, Scherer SW** 1998 Variation in the AU(AT)-rich element within the 3′-untranslated region of PPP1R3 is associated with variation in plasma glucose in aboriginal Canadians. J Clin Endocrinol Metab 83:3980–3983
34. **Xia J, Scherer SW, Cohen PT, Majer M, Xi T, Norman RA, Knowler WC, Bogardus C, Prochazka M** 1998 A common variant in PPP1R3 associated with insulin resistance and type 2 diabetes. Diabetes 47:1519–1524.